Project Report – FY2006

Core Name: Applied Marine Genomics

Project Title: Functional Marine Genomics Project

Reporting Period: 1 September 2005 – 30 September 2006

Principal Investigators: Gregory Warr, Medical University of South Carolina

Associate Investigators: Louis Burnett and Karen Burnett, College of Charleston; Charles Cunningham, University of New Mexico; Paul Gross Medical University of South Carolina; Robert Chapman, South Carolina Department of Natural Resources; Jonas Almeida, MD Anderson Cancer Center; Matt Jenny, Woods Hole Oceanographic Institute.

Background and Rationale:

Increased human habitation and utilization of U.S. coastal regions has created a broad range of challenges to marine and estuarine organisms, habitats, and ecosystems that will alter the productivity, sustainability and aesthetic appeal of the coastal environment. In addition these changes have impacts on human health, through, for example, the transmission of infectious agents and toxic chemicals by seafood, and the presence of pollutants and harmful algal blooms in coastal waters. Alterations of the aquatic environment result from a wide variety of stressors, including pollution (point and non-point), global climate change, habitat modification, excessive nutrient enrichment, over-fishing, introduction of non-native species, and disease outbreaks. These stressors elicit a large range of responses at the molecular, cellular, organismal, population, and ecosystem levels. Managers of coastal ecosystems face difficult challenges in assessing accurately the impact of multiple stressors on the environment, and in early detection of environments that are in danger of degradation. Functional genomics is an established technology in biomedical science that has proven predictive power not available from traditional environmental toxicology. The rationale for a functional genomics approach to assessing the health of populations is that many (although not necessarily all) stressors encountered by an organism will perturb the dynamics of gene expression (as measured by messenger RNA levels) in characteristic ways, producing diagnostic and predictive "transcriptional signatures". Accurate and sensitive tools are needed to meet this challenge, and the Functional Genomics Group at HML is committed to their development.

The objectives of the overall research program in functional genomics are as follows:

1. To develop microarray tools that will permit, through "transcriptional signatures", the diagnosis of the multiple stressors to which the organism has been exposed. An important part of this objective will be to refine the microarray design through laboratory experiments to contain the "minimal" number of genes to evaluate oyster exposure and the associated response.

2. To assess the microarray as a tool for the diagnosis and prognosis of oyster exposure and health in the marine environment. Oysters will be sampled from field sites in the marine estuarine environment, and the results from the microarrays will be combined with those from traditional measures of oyster health (e.g. assays of hemocyte function; levels of glutathione and of lipid peroxidation) and environmental health (e.g. sediment contaminant levels, benthic community composition) to test the hypothesis that functional genomics techniques and information can provide a sensitive assessment of environmental health.

It is recognized that the number of potential stressors that could have been chosen is large, and that all possible concentrations and interactions cannot be assessed, especially when natural variables such as salinity, hypoxia, and temperature are planned for eventual inclusion. Thus, the initial challenge experiments will utilize single toxicant exposures at concentrations that are known from the literature to induce physiological responses under relatively standard environmental conditions (e.g., 25 ppt, 25^{o} C, 80% DO saturation). These data will be used to develop models of transcript profiles in response to exposure to individual metals.

The Functional Genomics Research Project will cooperate closely with two other groups within OHH in the conduct of challenge experiments. The first group is the "Marine Organisms as Disease Vectors Project", with whom we will conduct transcript profiling studies on oysters exposed to infection with *Vibrio spp*., to hypoxia, and to pH, singly and in combination. The "Marine Organisms as Disease Vectors Project" will conduct the physiological and microbiological observations that will be correlated with changes in transcript profiles. Second, the functional genomics project will also take advantage of the tidal creeks field program being conducted by the Monitoring and Assessment Core to collect natural oysters along with a large suite of environmental (water and sediment) quality parameters. The later specimens will be used to develop models of transcript profiles for "real world" oysters. The Functional Genomics Research Project will also collaborate extensively with researchers at other institutions. This collaboration includes working with the "Oyster Genome Consortium" to develop and utilize genomic resources for the oyster, and with Dr. Sylvain De Guise of the University of Connecticut to study the transcriptomic response of the oyster to infection with the parasite *Perkinsus marinus*.

Accomplishments:

- A validated cDNA microarray containing both oyster genes (both *C. virginica* and *C. gigas*) has been developed, in collaboration with the international Oyster Genome Consortium. This tool is being made available to, and will be widely used by, the oyster research community and marine ecosystem managers to assess the health status of oysters and their responses to environmental stressors.
- Two laboratory-based experiments utilizing the microarray to understand the response of the oyster to stressors have been conducted. In the first, 216 oysters were exposed to single and multiple exposures of copper, zinc and cadmium, at concentrations ranging from low to high. RNA was isolated from gills and

hepatopancreas and hybridized to the microarrays, and actual metal burden, glutathione levels and lipid peroxidation were measured for each oyster. In addition, the Oysters as Disease Vectors Group (as referenced in the Report of this Group) measured a suite of physiological parameters, including hemocyte count, pO_2, pCO_2 and pH. In the second experiment, oysters challenged by sterile wounding and by wounding accompanied by exposure to heat-killed bacteria and fungi were conducted (in collaboration with Drs Cunningham and Jenny). RNA samples from these oysters have also been hybridized to the microarray. The results from the second study have been analyzed and are presented in detail in the Report of the Bioinformatics Core (Dr Robert Chapman), but in summary it is clear that the transcriptomes of control oysters, sterile wounded oysters, and septic wounded oysters can be reliably distinguished.

- Publications describing the generation of BAC libraries in C. virginica and C. gigas and the regulation of metallothionein genes of C. viriginica in response to metals exposure and immune challenge have been published. These articles will be of value to researchers in oyster physiology and to researchers in ecology by advancing our understanding of marine environmental science at a molecular and genomic level. Research presentations on oyster genomics and their transcriptomic responses to stressors were made at the Tenth Congress of the International Society for Developmental and Comparative Immunology held in Charleston, SC in July 2006. Drs Chapman and Warr attended the Workshop on Biodiversity and Human Health, organized by NOAA, EPA and Yale University and held at the Smithsonian Institution on September 14 and 15. At this Workshop they presented the approach developed by the OHHI team at Hollings Marine Laboratory to understanding the biological response of the environment to multiple stressors.

Publications:

Cunningham C, Hikima J, Jenny MJ, Chapman RW, Fang G-C, Saski C, Lundqvist ML, Wing RA, Cupit PM, Gross PS, Warr GW, Tomkins JP. (2006) New Resources for Marine Genomics: BAC libraries for the Eastern and Pacific oysters (*Crassostrea virginica* and *C. gigas*) Marine Biotechnology. In Press

Jenny MJ, Warr GW, Ringwood AH, Baltzegar DA, Chapman RW. (2006) Regulation of Metallothionein Genes in the American Oyster (*Crassostrea virginica*): Ontogeny and Differential Expression in Response to Different Stressors. Gene, 379, 156-165.

Presentations:

Chapman RW, McKillen DJ, Trent HF, Chen YA, Almeida JS, Gross PS, Warr GW, Robalino J, Jenny M, Cunningham C. Ecogenomics: Analytical Challenges and Potential Solutions. Tenth Congress of the International Society for Developmental and Comparative Immunology, Charleston, SC, July 2006

Cunningham C, Jenny MJ, Chen YA, Trent HF,m McKillien DJ, Almeida JS, Chapman RW, Warr GW. Design and characterization of a multi-species oyster cDNA microarray

and its use in transcript profiling of immune challenged *Crassostrea virginica*. Tenth Congress of the International Society for Developmental and Comparative Immunology, Charleston, SC, July 2006

Application/Technology Transfer:

1.0 Scientific Research and Application

Microarrays for the transcriptomic assessment of the health and response to environmental stressors of 3 species of marine organism (oyster, shrimp and dolphin) have been developed. Additional microarrays for grass shrimp and the sea urchin are under development. These transcriptomic tools are being used in "proof of concept" studies to quantify and predict how human activities (e.g. coastal habitation, recreational and industrial activities) impact the marine environment and the potential hazards to human health posed by altered marine ecosystems.

2.0 Public Information and Outreach

Oyster and shrimp cDNA microarrays have been delivered to the national and international community of oyster and shrimp health researchers. The marine genomics website provides a publicly accessible database and set of analytical tools for understanding the genomics of selected marine organisms (including the American oyster, Atlantic white shrimp, grass shrimp and the Atlantic bottlenose dolphin) and assessing their health using transcriptomic methods.

3.0 Capacity Building

Additional robotic equipment necessary for the generation and use of genomic tools has been acquired and commissioned, and staff have been trained in the effective and accurate use of all robotic instruments and in the experimental methods and analytical techniques necessary for functional genomic science. Genomics services are provided on a space-available and cost-recovery basis to other investigators (both inside and outside the Hollings Marine Laboratory) whose genomics research is compatible with the goals of the OHH initiative.

Project Abstract:

The basic tools and methods have been developed for investigating oyster transcriptomic and proteomic responses to challenges and stressors present in the marine environment, such as changes in salinity, temperature, oxygenation, pollution and infectious diseases. Data derived from the use of microarrays in oyster, shrimp and dolphin suggests that transcriptomic approaches will be a highly sensitive and informative method for assessing the interaction of marine organisms with multiple stressors. The completion of these studies will provide methods for the accurate and effective assessment of the health status of the marine environment and of some potential hazards that it might pose to the health of humans consuming fish and shellfish, living beside the ocean, and using it for recreation or commerce.

Unresolved issues:

- The bioinformatics capacity (in terms of both personnel and computing capacity) to manage and analyze extremely large datasets continues to be a major challenge for the Functional Marine Genomics Projects that must be overcome. A major challenge identified in the previous Progress Report, i.e. the translation of the research from laboratory-scale populations to broad, living ecosystems is currently being addressed in a direct test of oysters sampled from field sites.

Budget Report:

As of 31 August 2006, total expenditures and commitments totaled:

Personnel:	$222,605
Cont. Services	$450
Supplies	$5,486
Travel	$3,767
Trainee Stipends	$22,040
Total D/C	$254,348
F&A	$66,051
Total	$320,399

Breakdown of expenditures by budget year

Fiscal Year 2005:

Personnel	$ 92,431
Supplies	2,930
Travel	333
F&A	24,881
Total	$120,575

Fiscal Year 2006:

Personnel	$151,906
Supplies	2,556
Travel	3,884
F&A	41,170
Total	$199,516

Fiscal Year 2007 (to date - runs through Sept. '07):

Personnel	$ 21,006
F&A	5,066
Total	$ 26,072